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IMPROVING THE OCULAR ABSORPTION OF PHENYLEPHRINE

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ABSTRACT

An oxazolidine prodrug of phenylephrine and the base form of phenylephrine were synthesized, suspended in sesame oil, and tested for mydriatic activity against phenylephrine HCl. The HCl salt was formulated as a viscous aqueous solution and as a sesame oil suspension. A dosing volume of 10 µl was instilled into rabbit eyes and the pupillary diameter was measured over time. A 0·045 M prodrug suspension was judged equal in mydriatic activity to a 0·45 M viscous solution of phenylephrine HCl with the exception that the time of maximum response occurred 60 min earlier with the prodrug.

When phenylephrine base was suspended in sesame oil at 0·045, 0·12, and 0·45 M, the mydriatic activity was also greater than equimolar suspensions of phenylephrine HCl. The pH of tear fluids was also measured over time and found to rise 1·1, 0·70, and 0·30 pH units for 0·45, 0·12, and 0·045 M suspensions of the base form but remain unchanged when phenylephrine HCl was instilled in the rabbit eye. The greater activity associated with the base form of phenylephrine was judged a result of the change in pH to favour the absorption of phenylephrine. This latter approach should be applicable to either weak acids or weak bases with pK_a values outside of the normal pH range (7–8) of the tears and in concentrations greater than 0·045 M suspended in a non-aqueous vehicle.

KEY WORDS Phenylephrine Mydriasis Rabbits Prodrug

INTRODUCTION

Phenylephrine HCl is a very hydrophilic drug used in the eye for its mydriatic and capillary decongestion effects. Because of its hydrophilic characteristics, it poorly penetrates the epithelium of the cornea,[1] therefore, a relatively high concentration, 2·5 or 10 per cent must be applied to the eye topically to achieve a potent effect. As a result of applying a large dose which is poorly absorbed, significant systemic side-effects occur in some individuals ranging from transient hypertension, syncope, and in a few reported cases, myocardial infarction leading to death.[2–4]

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Clearly there is a need to reduce the systemic side-effects of phenylephrine. Theoretically this could be accomplished by increasing the ocular absorption and thereby reducing systemic absorption. However, the reduction in systemic side-effects is an indirect effect of improved ocular absorption. This is exemplified by the fact that even though ocular absorption may double from 2 to 4 per cent, systemic absorption decreases from 98 to only 96 per cent, which is not a significant decrease with respect to systemic side-effects. Nevertheless, by increasing the absorption twofold, the dose can be reduced by one-half to achieve the same therapeutic effect and in this manner systemic effects can be reduced substantially.

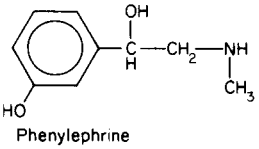
Because phenylephrine HCl is sufficiently potent and because an improvement in its potency would likewise lead to more potent cardiovascular side-effects, modification of the overall structure is not the best approach to solving the problem. Drug delivery methods, i.e., formulation modification or prodrug synthesis, are particularly well suited to improving absorption yet maintaining the drug's potency.

In these studies we examined the improvement in mydriasis using either a prodrug of phenylephrine or phenylephrine base, each formulated as a suspension in sesame oil.

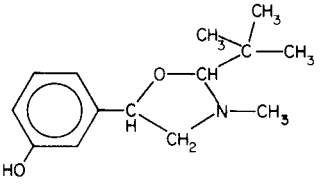
MATERIALS AND METHODS

Synthetic procedures

Oxazolidine prodrug of phenylephrine. (R)-(-)phenylephrine base (0·83 g, 5 mmol), pivaldehyde (0·55 ml, 5 mmole), and benzene (100 ml) were refluxed together with stirring for 60 h under a Dean and Stark trap. The solvent was removed under reduced pressure to yield the oxazolidine prodrug, 2-*t*-butyl-3-methyl-5-(*m*-hydroxyphenyl)-1,3-oxazolidine. The prodrug was recrystallized from benzene and dried. Figure 1 compares the



Phenylephrine



Oxazolidine derivative

Figure 1. Structural comparison of phenylephrine and an oxazolidine prodrug of phenylephrine

chemical structure of phenylephrine and the oxazolidine prodrug. A general synthetic procedure was previously described for oxazolidines from beta-aminoalcohols.[5]

Phenylephrine base. Phenylephrine HCl (0·3 g, 1·47 mmole) was dissolved in 3 ml of water in a test tube to which was added 1 ml of ammonia TS. The interior of the test tube was rubbed with a glass rod to initiate the precipitation of the free base. The phenylephrine was separated by filtration and washed with about 1 ml of ice-cold water and finally dried over silica gel for 16 h.[6]

Formulations

Table 1 lists the ingredients used in preparing the viscous solution and the sesame oil suspensions.

Table 1. Composition of aqueous and sesame oil formulations used in the rabbit eye

		% w/v
*Viscous vehicle**		
KH_2PO_4 (anhydrous monopotassium phosphate)		0·75
Na_2HPO_4 (anhydrous disodium phosphate)		0·175
Benzalkonium chloride, N.F.		0·01
Methylcellulose, U.S.P.‡		0·85
Distilled water	qs	100·0
Oil vehicle†		
Poloxamer P103		0·01
Methylparaben		0·05
Propylparaben		0·01
Chlorobutanol		0·25
Sesame oil	qs	100·0

*L-Phenylephrine HCl, U.S.P. (0·45 M) was dissolved in the viscous vehicle.
†Phenylephrine (0·045, 0·12 and 0·45 M), phenylephrine HCl (0·45 M), and phenylephrine oxazolidine (0·45 and 0·045 M) were suspended in the sesame oil vehicle.
‡400 Centipoise.

The viscous solution of phenylephrine HCl (0·45 M) was prepared by first heating 60 ml of distilled water to about 80° and then adding methylcellulose (Hercules, Wilmington, Del) powder. The solution was stirred until all the powder was wetted. The heated solution was then placed in an ice bath and cooled until the methylcellulose was fully hydrated. To slightly less than 40 ml

of distilled water, each ingredient was added until dissolved. The two solutions were mixed together and brought to 100 ml using distilled water (pH 5·75). The final viscosity of the viscous solution was 30 (± 2) centipoise using a Brookfield RV Viscometer (Stoughton, Mass).

A sesame oil (Sigma, St. Louis, MO) vehicle was used to suspend phenylephrine (0·45, 0·12, and 0·045 M), phenylephrine oxazolidine (0·45 M and 0·045 M) or phenylephrine HCl (0·45, 0·12, and 0·045 M). The parabens and chlorobutanol were dissolved in about 75 g of sesame oil. The oil was heated to about 40° to facilitate the dissolution of each ingredient. The Poloxamer P103 (Pluronic P103, BASF Wyandotte, Wyandotte Corp., Wyandotte, MI) was incorporated into the drug powder using a mortar and pestle. The remaining sesame oil was added to the wetted drug powder and triturated until an acceptable slurry was formed. The sesame oil containing the preservatives was added to the slurry to form the suspension.

Mydriatic measurements

Mydriasis was measured in the left eye of normal adult New Zealand rabbits (3–4 months old, 1·8 to 1·4 kg, either sex, $n = 6$). A flood of diffuse light was placed at a fixed distance from the rabbit eye so that the initial pupil diameter prior to administering eye drops was about 3·5 mm. Changes in pupillary diameter were measured from photographs taken with a 35 mm single lens reflex camera (Pentax 1000, Asahi Opt. Co., Japan) equipped with a close-up lens (Kenko Co., Japan). Pupillary diameters were measured from time 0 through about 5 h. A dosing volume of 10 µl was administered to the left eye of a group of six rabbits. A rest period of at least 3 days was allowed between instillations of each formulation in the same group of rabbits.

Hyrolysis rate studies

A series of experiments were conducted at 35° to determine the apparent first order hydrolysis rate constant for phenylephrine oxazolidine at pHs 1–7·4. The progress of hydrolysis of the prodrug was followed by measuring the production of pivaldehyde subsequently trapped with thiosemicarbazide (pH < 4) or semicarbazide (pH 4–7·4). The resulting formation of the carbazone derivative was followed using UV spectroscopy at 262 and 235 nm (8450A UV/VIS Hewlett-Packard, Chicago, Ill). The prodrug was dissolved in either pH 1, 2, 3, 4, 5, 6, 7, or 7·5 buffered aqueous solutions. The buffers used were hydrochloric acid, formate, acetate, and phosphate solutions. The carbonyl trapping reagent was included in the buffer solutions at a concentration of $3{\cdot}1 \times 10^{-3}$ M. The initial concentration of oxazolidine prodrug was about $1{\cdot}8 \times 10^{-4}$ M. A volume of 3 ml was used for the reaction which was monitored directly in the absorbance cell. The hydrolysis displayed pseudo-first-order kinetics such that the hydrolysis rate constant could be determined from the slope of a linear ln plot of the absorbance of carbazone remaining to be formed over time.[7]

Tear pH measurements

In separate experiments but using the same rabbits in which phenylephrine base or the HCl salt were compared, the pH of the tears was monitored over time. Paper impregnated with pH indicator (Color**pH**ast indicator sticks, E. Merck, Darmstadt, F. R. Germany) was carefuly placed in the conjunctival sac of the dosed rabbit eye for about 10 s until a colour change occurred. By this method, the tear pH was estimated at various time intervals within 0·2 units between pH 6·8–7·8 and 0·3 units between pH 7·8–9·5.

Octanol/water distribution coefficient

The distribution coefficient was measured at pH 7·4 according to the method of Bundgaard and Johansen.[5]

RESULTS AND DISCUSSION

Oxazolidine prodrug

The identification of the oxazolidine prodrug of phenylephrine was based upon the following results:

IR (KBr) – 2970, 2870, 1600, 1450, 1400, 1375, 1265, 1220, 1160, 1120, 1070, 970, 860, 780 and 700 cm^{-1}.
NMR (CDCl) delta = 7·2–6·6 (m, 4H), 5·1–4·8 (m, 1H), 4·0 (bs, 1H), 3·5–2·8 (m, 2H), 2·5 (bs, 3H), (bs, 9H) ppm.
Elemental analysis for $C_{14}H_{21}NO_2$: C, 71·46; H, 8·99; N, 5·95. Found: C, 71·71; H, 8·87; N, 5·93.
The percent yield for the synthesis of the prodrug was 92 per cent.

Figure 2 compares the mydriatic response in rabbit eyes for equimolar doses (0·45 M) of phenylephrine HCl and the oxazolidine prodrug. The prodrug shows a significantly greater effect than phenylephrine HCl which can be attributed to the greater lipophilicity of the prodrug. Its log octanol/pH 7·4 buffer distribution coefficient was $1·38 \pm 0·13$ which is favourable for rapid corneal absorption for many ophthalmic drugs.[1,8]

Figure 3 shows the degree to which a topically instilled dose of the prodrug can be reduced based upon the mydriatic response in the rabbit eye. The mydriatic profile for the 0·045 M prodrug in Figure 3 is similar to the profile obtained for the 0·45 M viscous phenylephrine HCl with the exception that the time of maximum response is 60 min earlier for the prodrug. This occurs because of the more rapid corneal penetration of the prodrug.

Table 2 shows the rapid rate of hydrolysis for the conversion of the prodrug to phenylephrine at *in vitro* pHs of 1 to 7·4. The conversion half-lives were 6–13 min. The rate would be expected to be at least as rapid in the eye. For the most part conversion likely occurs in the cornea and not in the tear fluids

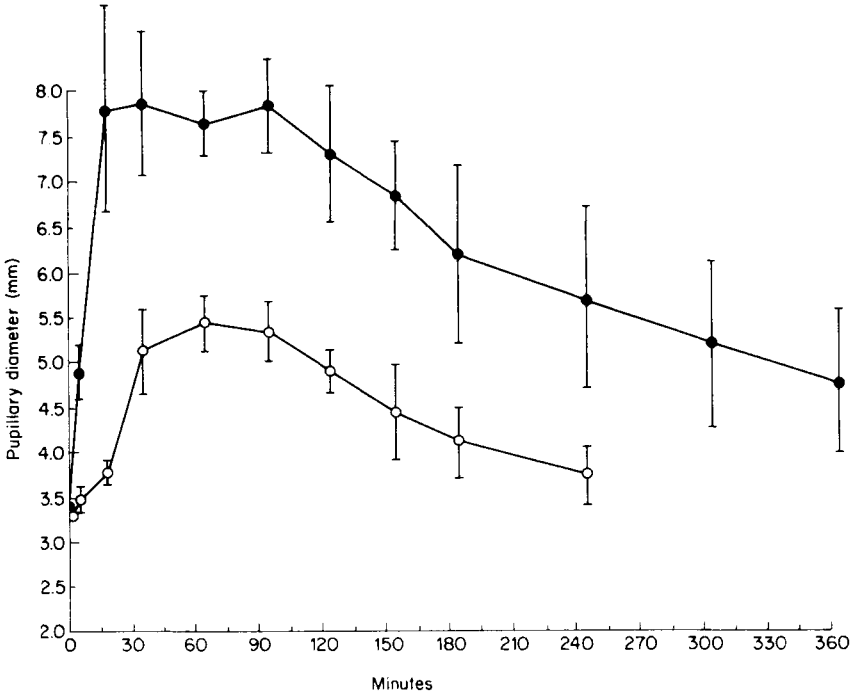


Figure 2. A comparison of pupillary diameters in rabbit eyes ($n = 6$) for phenylephrine oxazolidine (—●—, 0·45 M) and phenylephrine HCl (—○—, 0·45 M) each formulated as an oil suspension and dosed in a volume of 10 μl

or the difference in bioavailability would not be as large as observed in Figure 2. Because of the instability of the prodrug, it had to be formulated as a sesame oil suspension.

A pivalic acid ester (on the meta position of the benzene ring of phenylephrine) had been previously investigated as a lipophilic drug.[9] Likewise, it showed about a tenfold improvement in the mydriatic activity when compared to phenylephrine HCl. However, there was some question as to whether it was a true prodrug or not.[10] The pivalate ester of phenylephrine depended upon esterase activity for rapid conversion to phenylephrine. Rabbit eyes were pretreated with echothiophate for 2 or 7 days prior to instillation by either phenylephrine or the pivalate ester. The conversion was expected to be inhibited by echothiophate pretreatment, however, the authors found that there was no difference in mydriatic activity in the drug or prodrug suggesting to them that the prodrug had substantial intrinsic alpha-adrenergic activity.[10]

Since the oxazolidine prodrug of phenylephrine increases the bulk considerably on the amine functionality, it was hypothesized that the

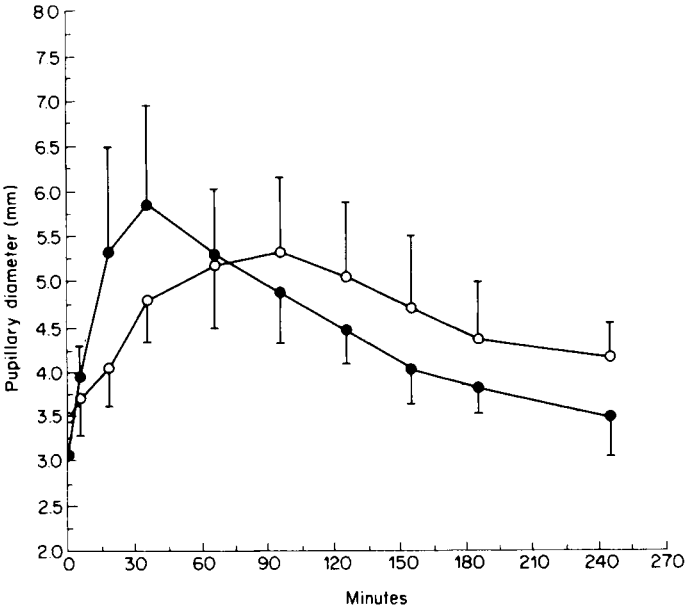


Figure 3. A comparison of pupillary diameters in rabbit eyes ($n = 6$) for phenylephrine oxazolidine (—●—, 0·045 M oil suspension) and phenylephrine HCl (—○—, 0·45 M aqueous viscous solution) each dosed in a volume of 10 µl

oxazolidine prodrug would be devoid of alpha-adrenergic activity.[11] Also, because of its rapid hydrolysis at pH 7–7·5, conversion to phenylephrine would not depend on esterase activity within the cornea. Although rapid conversion in an aqueous system at any pH would create a stability problem for a prospective product, it could be overcome by using a non-aqueous vehicle.

Table 2. Half-lives for the hydrolysis of phenylephrine oxazolidine to phenylephrine

pH	$t_{1/2}$ (min)*
1	11·9
2	10·4
3	9·2
4	7·9
5	6·3
6	7·7
7	8·7
7·4	13·2

*Calculated from first-order hydrolyis rate constants.

Phenylephrine base

Figures 4, 5, and 6 give the mydriatic results for the phenylephrine base and phenylephrine HCl, both formulated as sesame oil suspensions and compared at three different equimolar concentrations. At 0·45 M the base form showed a much greater mydriatic effect than the HCl salt. This was attributed to the

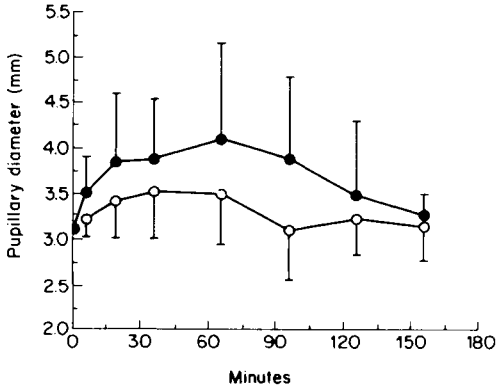


Figure 4. A comparison of pupillary diameters in rabbit eyes ($n = 6$) for phenylephrine base (—●—, 0·045 M) and phenylephrine HCl (—○—, 0·045 M) each formulated as an oil suspension and dosed in a volume of 10 µl

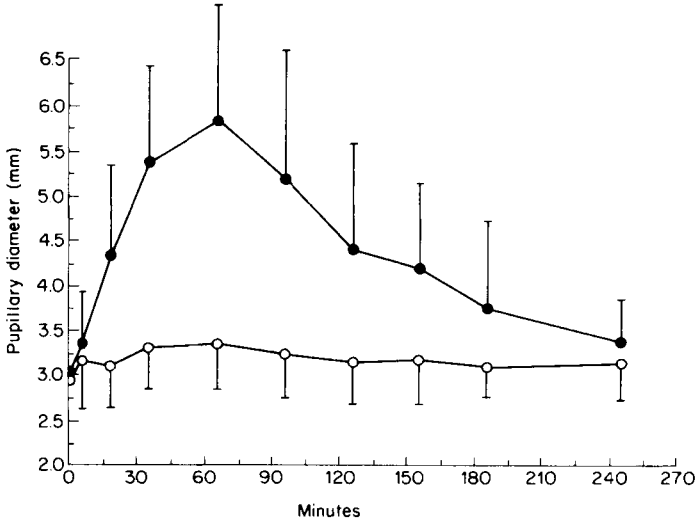


Figure 5. A comparison of pupillary diameters in rabbit eyes ($n = 6$) for phenylephrine base (—●—, 0·12 M) and phenylephrine HCl (—○—, 0·12 M) each formulated as an oil suspension and dosed in a volume of 10 µl)

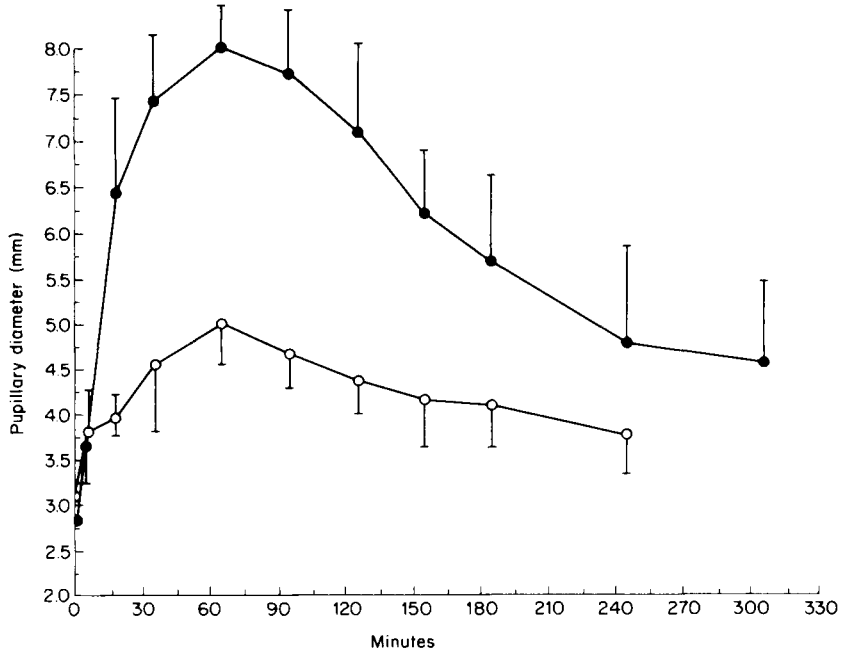


Figure 6. A comparison of pupillary diameters in rabbit eyes ($n = 6$) for phenylephrine base (—●—, 0·45 M) and phenylephrine HCl (—○—, 0·45 M) each formulated as an oil suspension and dosed in a volume of 10 µl

fact that the base form raised the pH of the tear fluids and thereby promoted its own absorption.

Evidence for the rise in tear pH is given in Table 3. For the base form, the increase in pH units compared to just before the instillation of the dose was 1·1, 0·70, and 0·30 for 0·45, 0·12, and 0·045 M concentrations, respectively. The increase in the mydriatic effect of the base form over the HCl salt was greatest for 0·45 M and least for the lowest concentration, 0·045 M, indicating the improvement in effect also depends on concentration as well as which form of the drug is used. When compared to baseline ($t = 0$) pH values, only pH measurements at 10, 20, and 30 min for 0·5 and 0·12 M concentrations of the base form were statistically significant (t-test, $p < 0{\cdot}05$). Surprisingly, the HCl salt did not lower the pH of the tears over time.

The use of the unionized form of the drug suspended in a non-aqueous vehicle should be applicable to either weak bases or weak acids with pK_a values outside the normal pH range of tears (7–8) and in concentrations greater than approximately 0·045 M (or approximately 1 per cent w/v).

The oxazolidine prodrug and the base form of phenylephrine, both formulated in sesame oil, permit a significant reduction in the instilled dose

Table 3. Mean pH (± S.D.) of tears after instillation of phenylephrine or phenylephrine HCl to the right eye of rabbits

Time (min)	0·45 M		0·12 M		0·045 M‡	
	Base	HCl	Base	HCl	Base	HCl
0	7·8 (0·0)	7·7 (0·19)	7·6 (0·05)	7·5 (0·15)	7·6 (0·13)	7·7 (0·18)
10	8·6 (0·24)*	7·6 (0·16)	8·0 (0·16)*	7·7 (0·13)	7·9 (0·25)	7·7 (0·21)
20	8·9 (0·05)*	7·5 (0·10)	8·3 (0·24)*	7·6 (0·17)	7·8 (0·31)	7·5 (0·15)
30	8·5 (0·26)*	7·6 (0·21)	8·3 (0·22)*	7·7 (0·35)	7·7 (0·25)	7·6 (0·15)
40	8·6 (0·40)†	7·6 (0·05)	8·0 (0·30)	7·8 (0·10)	7·6 (0·13)	7·6 (0·08)
50	8·2 (0·41)	7·6 (0·13)	7·8 (0·10)	7·6 (0·13)		
60	8·2 (0·34)	7·6 (0·13)	7·7 (0·15)	7·6 (0·14)		
70	8·0 (0·19)	7·7 (0·12)				
80	7·9 (0·10)	7·7 (0·10)				

*$p < 0.05$ (all values compared to time = 0).
†$p < 0.1$ (compared to time = 0; all other pH values were statistically non-significant).
‡Average of 6 rabbit eyes; all other formulations were tested in 4 rabbit eyes.

without a loss of potency. Either approach should significantly reduce the need for instillation of 10 per cent in the eye and thus decrease the systemic side-effects of phenylephrine.

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